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Morphology and chemistry of Dufour glands in four ectoparasitoids: *Cephalonomia tarsalis, C. waterstoni* (Hymenoptera: Bethylidae), *Anisopteromalus calandrae*, and *Pteromalus cerealellae* (Hymenoptera: Pteromalidae)

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Abstract

The venom apparatus of four hymenopterous parasitoids, including two bethylids, *C. tarsalis* (Ashmead) and *C. waterstoni* (Gahan), and two pteromalids, *A. calandrae* (Howard) and *P. cerealellae* (Ashmead), were removed and the associated Dufour glands characterized with respect to their external morphology and chemistry. Dufour glands in all four species have a characteristic translucent appearance that apparently results from their lipid content. The stalked Dufour glands of *C. tarsalis* and *C. waterstoni* are pear-shaped and have overall lengths of approximately 0.2 and 0.15 mm, respectively. The thin venom glands are bifurcate and insert through a fine duct into the transparent ovoid- to pear-shaped venom reservoir in these bethylids. In *A. calandrae* and *P. cerealellae* the Dufour glands are elongated, tubular structures of ca. 0.35 and 0.8 mm in length, respectively, that constrict to a short stalk that empties into the common oviduct. The venom glands in these pteromalids are simple elongated structures that insert into the sac-like venom reservoir through a fine duct. The chemistry of the volatile contents of the Dufour gland in these four species differs considerably. *C. tarsalis* Dufour glands contain the same hydrocarbon components as found on the cuticle of this species (Ann. Entomol. Soc. Am. 91:101–112 (1998)), and no other chemicals. The Dufour glands of *C. waterstoni* also contain only hydrocarbons, most of which are the same as the cuticular hydrocarbons (Ann. Entomol. Soc. Am. 85:317–325 (1992)), but in addition the Dufour gland contains ca. 3% of a mixture of 2,17- and 2,19-dimethyl C_{23}. *A. calandrae* Dufour gland chemistry is somewhat more complex than that of either of the two bethylids, but like the bethylids, only hydrocarbons are present. The carbon number range is from C_{30} to C_{39} and consists of a mixture of *n*-alkanes (C_{30}-C_{38}); 3-, 5-, 7-, 9-, 11-, 12-, 13-, 14-, 15- and 17-methyl alkanes; 3,7- and 3,11-dimethyl alkanes; 5,9- and 5,17-dimethyl alkanes; 7,11-, 9,13-, 13,17-, 14,18- and 15,19-dimethyl alkanes; 3,7,11- and 3, 9,15-trimethyl alkanes; and 3,7,11,15-tetramethyl alkanes. The cuticular hydrocarbons of this species have not been previously reported, but they are the same as the Dufour gland hydrocarbons. The Dufour glands of *P. cerealellae* contain both hydrocarbons and two long-chain aldehydes. Most of the hydrocarbons are identical to those found on the cuticle of this species (Ann. Entomol. Soc. Am. 94:152–158 (2001)), but in addition, 5,9-dimethyl C_{27}, 5,13-, 5,17- and 5,19-dimethyl C_{35}, 12- and 14-methyl C_{36}, 12,16- and 13,17-dimethyl C_{36}, 13-methyl C_{37} and 13,17-dimethyl C_{37} are present. The two aldehydes detected in glands from *P. cerealellae* are *n*-tetracosanal (C_{23}CHO) and *n*-hexacosanal (C_{25}CHO).

Keywords: Parasitoids; Ectoparasitoids; Venom apparatus; Dufour gland; Hydrocarbon; Aldehydes; Semiochemistry; Morphology; Stored products; Biological control

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1. Introduction

Dufour gland secretions in Hymenoptera contain a broad array of chemicals that have been implicated in a number of physiological and semiochemical functions (Blum, 1985; Haynes and Birch, 1985; Ali and Morgan, 1990). The Dufour gland secretions of many aculeates, including ants, bees and wasps, are particularly well studied (Billen and Morgan, 1998), whereas much less information is available for parasitic Hymenoptera (Jervis and Kidd, 1996; Quicke, 1997). There is evidence that Dufour gland secretions in some braconids have oviposition deterrent effects (Vinson and Guillot, 1972; Guillot et al., 1974) and serve as mating pheromones (Syvertsen et al., 1995), and that components of the Dufour gland secretion can mediate oviposition responses in an ichneumonid (Mudd et al., 1982; Marris et al., 1996). However, there is no information available on the Dufour glands and their chemical contents in either the Bethylidae or Pteromalidae, two families that contain important ectoparasitoids associated with major agricultural pests. In this report we present our findings on the gross morphology and chemistry of volatile components of Dufour glands in *C. tarsalis* (Ashmead) and *C. waterstoni* (Gahan) (Bethylidae) and in *A. calandrae* (Howard) and *P. cerealellae* (Ashmead) (Pteromalidae). These four cosmopolitan species are important parasitoids that attack the major insect pests of stored grain in North America.

2. Materials and methods

2.1. Insects

Adult female parasitoids ca. one week old were obtained from stock cultures maintained in our laboratory. *C. tarsalis* and *C. waterstoni* were reared at 30 °C and 55% RH on immatures of the rusty grain beetle (*Cryptolestes ferrugineus* (Stevens)) and the saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.)), respectively. *A. calandrae* was reared at 25 °C and 55% RH on immature rice weevils (*Sitophilus oryzae* (L.)) maintained on wheat and *P. cerealellae* was reared at 30 °C and 55% RH on immatures of the cowpea weevil (*Callosobruchus maculatus* (F.)) in cowpeas. The thin, ant-like bethylids are considered to be 'minute' parasitoids with lengths of approximately 2 mm for female *C. tarsalis* and 1.8 mm for *C. waterstoni*. The pteromalids, while quite small, are more 'robust' with overall lengths of 2 mm for *A. calandrae* and 3 mm for *P. cerealellae*.

2.2. Dissection procedure and sample preparation

Adult wasps were chilled on ice and dissected with ultra-fine forceps under 0.9% NaCl containing 0.05% Triton-X100 in a black depression plate. Lifting and removing several tergites exposed abdominal organs. By carefully removing the hind- and midgut, the terminal ganglion and the reproductive system, the Dufour gland and venom apparatus (venom reservoir and associated venom glands) could be observed. The venom apparatus of each species is located at the base of the ovipositor/stinger. In the bethylids, the ovipositor base is located near the tip of the abdomen, whereas in the pteromalids the base of the ovipositor is located near the center of the ventral abdomen. By separating the several sheaths and stylets that make up the ovipositor, the Dufour gland and its duct that inserts into the oviduct could be exposed. For gas chromatography/mass spectrometric (GC/MS) analysis of the individual gland, the Dufour gland was pinched off at its duct, rinsed briefly in water and macerated onto a solid phase micro-extraction (SPME) fiber insert. When individual glands so analyzed gave insufficient signal for structural identification, groups of pooled Dufour glands were analyzed by macerating dissected glands onto 1×3 mm pieces of Whatman #2 filter paper. Each filter was placed in a 50 μl insert in a glass GC vial with a Teflon-lined crimp cap. Samples were stored at -80 °C until analyzed. Photographs of the venom apparatus and associated Dufour gland of *A. calandrae*, *C. tarsalis*, and *P. cerealellae* were taken through a stereo microscope equipped with a 35 mm camera. Gland size was measured with an ocular micrometer at $50\times$.

2.3. Chemical analyses

Electron impact mass spectral analyses were conducted by using a Hewlett-Packard 5790 A gas chromatograph (GC) (Hewlett-Packard, Inc., San Fernando, CA) containing a DB-5 bonded phase capillary column (15 m long, 0.25 mm inside diameter) (J and W Scientific, Folsom, CA) connected to a Hewlett-Packard 5970 mass selective detector (MSD) and a Hewlett-Packard 9133 data

system. Ultrapure helium was the carrier gas, with a column head pressure of 0.75 kg/cm^2. Mass spectra were obtained at 70 eV. Analyses were conducted using temperature programming, with an initial temperature of 100 °C, a final temperature of 320 °C, program rate of 5 °C/min, and 20 min final hold period. The splitless injector was set at 275 °C and the GC/MS interface was at 280 °C. Retention times of each hydrocarbon component and equivalent chain length values (ECL) were obtained by comparison with known *n*-alkane standards (Howard et al., 1978). Individual components in the total ion scanning mode were identified from their characteristic EI-MS fragmentation patterns (Jackson and Blomquist, 1976; Nelson, 1978) in conjunction with equivalent chain length values. Positional isomers of alkenes were identified by comparison to ECL values of known alkenes from previously identified cuticular hydrocarbon samples. Each isomer is nearly base-line separated from other isomers of the same carbon number and the ECL values are highly reproducible. Individual Dufour gland analyses used a 7 μm polydimethylsiloxane bonded phase fiber in a Supelco SPME holder. Absorbed lipids were analyzed by GC-MS using the same parameters as listed above, with the exception that the fiber was desorbed for 2 min at 280 °C with the septum purge closed before beginning the temperature program.

2.4. Statistical analyses

Individual total ion current values for chemicals identified from GC-MS runs were converted into percentage values and means and standard deviations obtained. All species except *C. waterstoni* were analyzed by SPME and 3 replicate runs were obtained. For *C. waterstoni*, two replicates of 5 Dufour glands each on filter paper were suspended in 5 μl of iso-octane containing an internal standard of 57 ng/μl docosane and 2 μl removed and injected into the GC/MS. Individual peaks were identified and converted as above into percentage values and means and standard deviations obtained.

2.5. Voucher specimens

Voucher specimens of each of the four parasitoid species have been deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research, Manhattan, Kansas.

3. Results

3.1. Morphology

The Dufour glands of all four species have a distinctive appearance that is apparently associated with the oily nature of the gland contents. Those of the bethylids *C. tarsalis* (Fig. 1A,B) and *C. waterstoni* (not illustrated, but essentially identical to those of *C. tarsalis* except for size) are pear-shaped and have a much longer stalk than that of the pteromalids. They are also much smaller, ca. 0.2 mm in length in *C. tarsalis* and ca. 0.15 mm in length in *C. waterstoni*. The stalked venom reservoir of the bethylids is nearly transparent and is also pear-shaped in both species. The venom glands of *C. tarsalis* and *C. waterstoni* are bifurcate and insert through a fine duct into the venom reservoir (Fig. 1A,B). Note that the complete venom gland of *C. tarsalis* is not visible in the photomicrograph (Fig. 1A) In contrast, in the pteromalids *A. calandrae* and *P. cerealellae*, the Dufour gland is an elongated, tubular structure that constricts to a short stalk that inserts into the oviduct (Fig. 1C,D for *A. calandrae* and Fig. 1E, F for *P. cerealellae*). Lengths of the Dufour glands in these species are ca. 0.40 mm and 0.8 mm, respectively. The ducts of the Dufour glands of both species become closely associated with the base of the venom reservoir. However, because of their small size the exact insertion point of the Dufour gland ducts into the oviduct is difficult to determine. Although the venom reservoirs in *A. calandrae* and *P. cerealellae* are similar in size and shape (ca. 0.2 mm in height), the venom glands in *P. cerealellae* are about twice as long as those of *A. calandrae*, 2.6 mm compared with 1.2 mm, respectively. The venom glands terminate in a fine duct that becomes closely appressed to the base of the venom reservoir. In lateral view, the venom reservoir seems to 'slouch' somewhat onto the shaft of the ovipositor.

3.2. Dufour gland chemistry

The volatile components of the Dufour gland of *C. tarsalis* are all hydrocarbons and are nearly identical to those previously reported from the cuticle of this species (Howard, 1998). The Dufour

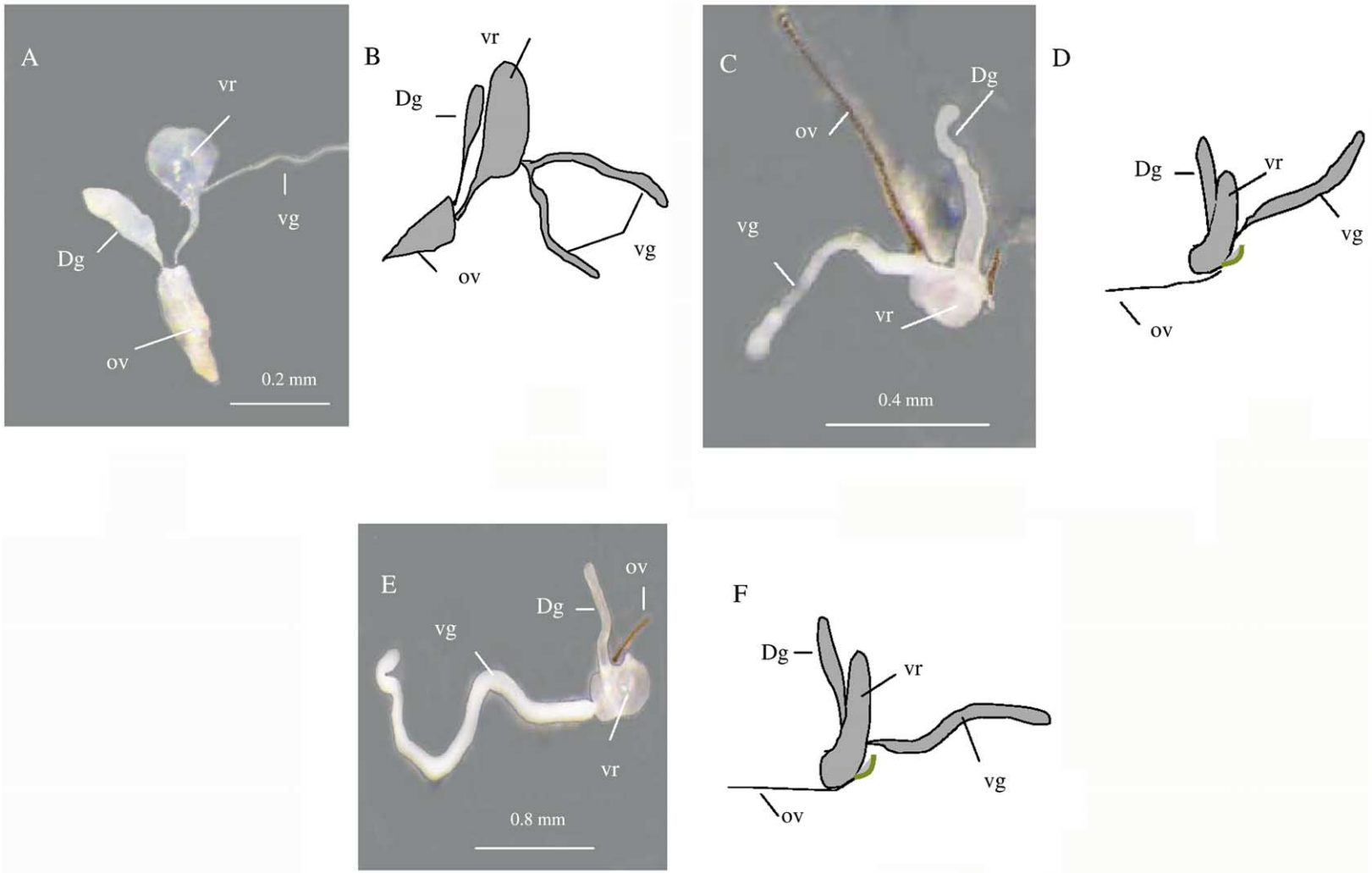

Fig. 1. Dissected venom apparatus of three ectoparasitoids: (A, B) photomicrograph of dorsal view and schematic drawing of glands in *C. tarsalis*; (C, D) photomicrograph of dorsal view and schematic drawing of glands of *A. calandrae*; (E, F) photomicrograph of dorsal view and schematic drawing of glands of *P. cerealellae*. Photomicrographs were imported in Adobe PhotoDeluxe© and backgrounds modified to enhance the contrast between the glands and the background. Figure legend: Dg Dufour gland, ov ovipositor, vg venom gland, vr venom reservoir. The schematic drawings illustrate the typical appearance of the venom apparatus in lateral view as seen in most dissections.

Table 1
Dufour gland chemistry and percentage composition in *C. tarsalis*

Compound	CN[1]	ECL[2]	Mean% (S.D.)($N=3$)
n-C_{23}	23	23.00	0.84 (0.04)
n-C_{24}	24	24.00	0.36 (0.13)
Z-11-$C_{25:1}$	25	24.68	0.22 (0.03)
Z-9-$C_{25:1}$	25	24.76	0.24 (0.06)
Z-7-$C_{25:1}$	25	24.82	0.99 (0.27)
n-C_{25}	25	25.00	10.93 (2.53)
5-MeC_{25}	26	25.50	1.13 (0.44)
Z-11-$C_{26:1}$	26	25.68	0.57 (0.12)
Z-9-$C_{26:1}$	26	25.75	0.51 (0.16)
Z-7-$C_{26:1}$	26	25.83	1.03 (0.18)
n-C_{26}	26	26.00	0.53 (0.13)
Z-11-$C_{27:1}$	27	26.69	11.42 (0.61)
Z-9-$C_{27:1}$	27	26.76	11.55 (1.45)
Z-7-$C_{27:1}$	27	26.82	26.24 (2.51)
n-C_{27}	27	27.00	4.64 (1.03)
5-MeC_{27}	28	27.51	1.09 (0.21)
Z-11-$C_{28:1}$	28	27.69	0.41 (0.08)
Z-9-$C_{28:1}$	28	27.74	0.48 (0.09)
Z-7-$C_{28:1}$	28	27.80	0.86 (0.12)
n-C_{28}	28	28.00	0.17 (0.05)
Z-11-$C_{29:1}$	29	28.69	5.93 (0.54)
Z-9-$C_{29:1}$	29	28.75	4.85 (0.52)
Z-7-$C_{29:1}$	29	28.82	8.99 (0.85)
n-C_{29}	29	29.00	1.98 (0.88)
5-MeC_{29}	30	29.53	0.34 (0.10)
$C_{30:1}$	30	29.71	0.20 (0.02)
5,17-+5,19-DiMeC_{29}	31	29.82	0.35 (0.08)
n-C_{30}	30	30.00	0.13 (0.03)
Z-11-$C_{31:1}$	31	30.67	0.40 (0.05)
Z-9-$C_{31:1}$	31	30.75	0.64 (0.06)
Z-7-$C_{31:1}$	31	30.81	0.34 (0.02)
n-C_{31}	31	31.00	1.12 (0.66)
$C_{32:1}$	32	31.70	0.02 (0.00)
n-C_{32}	32	32.00	0.05 (0.03)
$C_{33:1}$	33	32.70	0.11 (0.05)
n-C_{33}	33	33.00	0.32 (0.15)

[1] Carbon number.
[2] Equivalent chain length.

gland hydrocarbons and their percentage composition are given in Table 1 and include *n*-alkanes (C_{23}–C_{33}), 5-methyl alkanes (5-MeC_{25}–5-MeC_{29}), 5,X-dimethyl alkanes (5,17- and 5,19-diMeC_{29}) and a homologous series of Z-monoenes with double bonds at Δ^{11}, Δ^{9} and Δ^{7} ($C_{25:1}$–$C_{33:1}$). The monoenes constitute the major components (ca. 49% of the total) with the *n*-alkanes making up the next most abundant class (ca. 21% of the total). The ratio of the Δ^{11} and Δ^{9} alkene isomers are about equal with the Δ^{7} isomer being ca. twice the relative abundance of the other two isomers (Fig. 2a). Trace amounts of higher molecular weight components found on the cuticle of this species were not detected in Dufour gland extracts.

The components of the Dufour gland of *C. waterstoni* are all hydrocarbons, with the majority of the components being identical to those found on the cuticle of this species (Howard, 1992). The Dufour gland hydrocarbons and their percentage composition are given in Table 2 and include *n*-alkanes (C_{23}–C_{27}), 3-methyl alkanes (3-MeC_{23}–3-MeC_{25}), 5-methyl alkanes (5-MeC_{23}–5-MeC_{27}), 2,X-dimethyl alkanes (2,17- and 2,19-DiMeC_{23}), 5,X-dimethyl alkanes (5,17- and 5,19-DiMeC_{23}; 5,9-, 5,15-, 5,17 and 5,19-DiMeC_{25}), and three Z-monoenes (Δ^{11}-$C_{25:1}$, Δ^{7}-$C_{25:1}$ and Δ^{11}-$C_{27:1}$) (Fig. 2b). Fig. 3 is the EI-mass spectrum of a mixture of 2,17-dimethyl C_{23} (major component) and 2,19-dimethyl C_{23} (minor component). The ECL value for this mixture of compounds (23.95) demands that one of the two methyl branches be terminal and the other one at least 10 methylene units away (Pomonis et al., 1989). The predicted α-fragment ions as indicated on the structure in Fig. 3 are all present and in the correct even/odd relative abundance. The alternative structures, 2,4- and 2,6-dimethyl C_{23}, although having the same predicted ion fragments, would elute at an ECL of ca. 24.08 and have different even/odd ion fragment ratios (Pomonis et al., 1989). In addition to the hydrocarbons clearly identified, seven minor hydrocarbon components were detected, which were present in insufficient abundance to provide interpretable mass spectra All of the unknown components were not detected in the cuticular hydrocarbons of this species, and neither were the 2,X-dimethyl alkanes, the 5,9-DiMeC_{25} or the Δ^{7}-$C_{25:1}$. *n*-Alkanes are the major components (ca. 40% of the total) with nearly equal proportions of 5-methyl and 5,X-dimethyl hydrocarbons (ca. 32 and 31% each, respectively) making up the majority of the remaining hydrocarbons.

The Dufour gland chemistry of the pteromalid *A. calandrae* is somewhat more complex than that of either of the two bethylids. Like the bethylids, only hydrocarbons are present. The Dufour gland hydrocarbons and their percentage composition are given in Table 3. The carbon number range is from C_{30} to C_{39} and consists of a mixture of *n*-alkanes (C_{30}–C_{38}); 3-, 5-, 7-, 9-, 11-, 12-, 13-, 14-, 15-, and 17-methyl alkanes; 3,7- and 3,11-dimethyl alkanes; 5,9- and 5,17-dimethyl alkanes; 7,11-, 9,13-, 13,17-, 14,18- and 15,19-dimethyl alkanes; 3,7,11- and 3,9,15-trimethyl alkanes; and

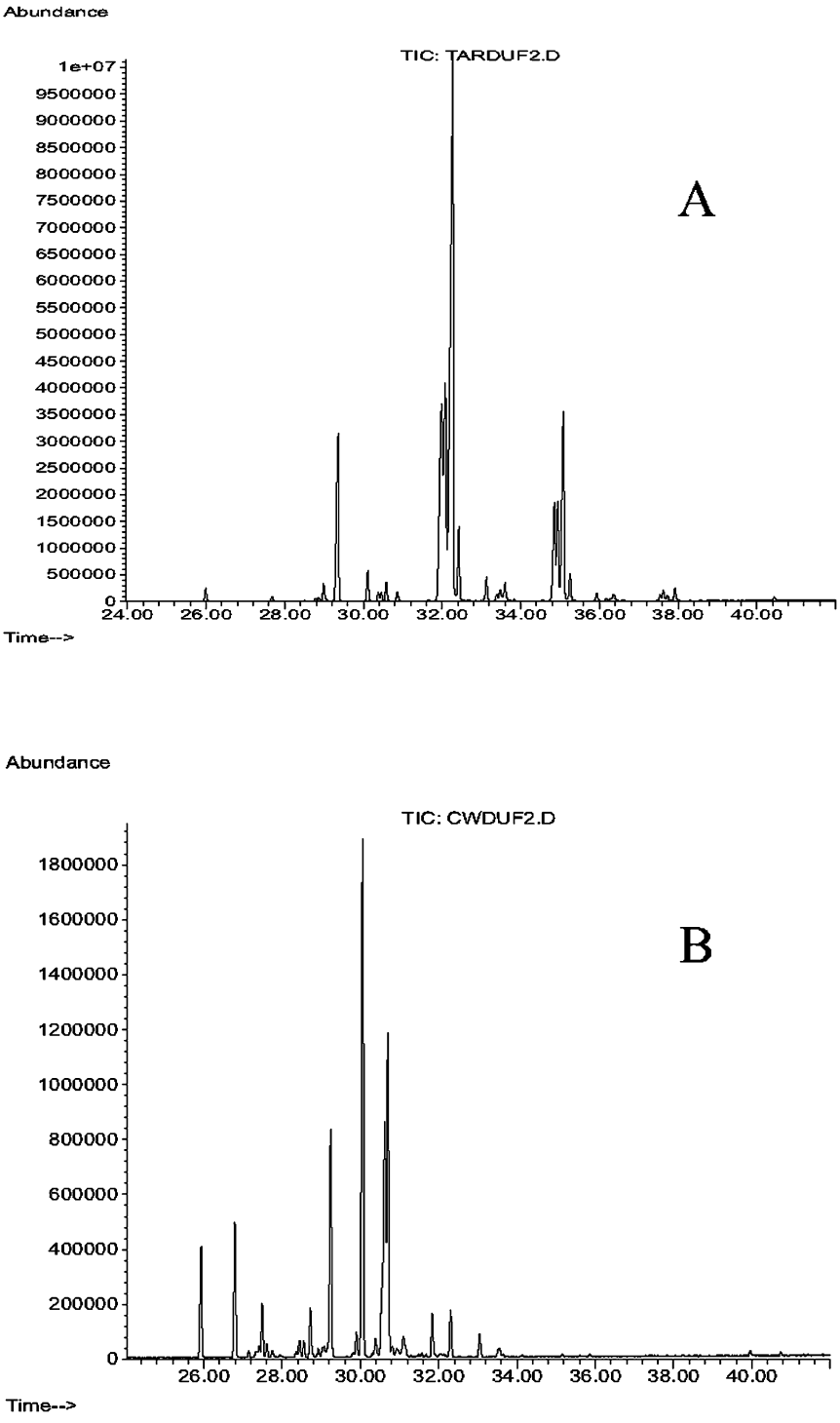

Fig. 2. Total ion trace of contents of the Dufour gland of two bethylids. (a) *C. tarsalis*; (b) *C. waterstoni*.

Table 2
Dufour gland chemistry and percentage composition in *C. waterstoni*

Compound	CN[1]	ECL[2]	Mean%(S.D.)($N=2$)
n-C_{23}	23	23.00	5.72 (0.22)
5-MeC_{23}	24	23.52	6.08 (0.68)
3-MeC_{23}	24	23.73	0.35 (0.08)
5,17-DiMeC_{23}	25	23.84	0.32 (0.05)
5,19-DiMeC_{23}	25	23.89	0.59 (0.05)
2,17-, 2,19-DiMeC_{23}	25	23.95	2.58 (0.06)
n-C_{24}	24	24.00	0.68 (0.04)
unk1	–	24.10	0.34 (0.02)
unk2	–	24.46	0.32 (0.00)
5-MeC_{24}	25	24.51	0.74 (0.11)
2-MeC_{24}	25	24.58	0.71 (0.08)
Z-11-$C_{25:1}$	25	24.68	2.56 (0.13)
Z-7-$C_{25:1}$	25	24.80	0.41 (0.01)
unk3	–	24.87	0.49 (0.04)
n-C_{25}	25	25.00	13.06 (1.69)
5-MeC_{25}	26	25.53	23.96 (1.84)
3-MeC_{25}	26	25.75	0.87 (0.03)
5,9- and 5,15-DiMeC_{25}	27	25.84	3.51 (0.09)
5,17-DiMeC_{25}	27	25.90	11.30 (0.33)
5,19-DiMeC_{25}	27	25.95	15.64 (0.53)
n-C_{26}	26	26.00	0.62 (0.10)
unk4	–	26.11	0.43 (0.11)
unk5	–	26.22	1.02 (0.06)
Z-11-$C_{27:1}$	27	26.70	2.27 (0.16)
unk6	–	26.83	0.21 (0.06)
n-C_{27}	27	27.00	3.05 (0.73)
5-MeC_{27}	28	27.53	1.70 (0.64)
unk7	–	27.93	0.49 (0.04)

[1] Carbon number.
[2] Equivalent chain length.

3,7,11,15-tetramethyl alkanes. Dimethyl alkanes are the major components (ca. 48% of the total), with monomethyl alkanes next at ca. 40% of the total. Trimethyl alkanes comprise ca. 3% of the total and tetramethyl alkanes make up ca. 4% of the total. The cuticular hydrocarbons of this species have not been previously reported, but they are the same as the Dufour gland hydrocarbons (Fig. 4a,b). Identification of the majority of the hydrocarbons was straightforward, as they have been previously described from other insects. The tetramethyl alkanes are less common, however, and have not been previously reported from Hymenoptera. Fig. 5 is the EI-mass spectrum of 3,7,11,15-tetramethyl C_{33} with an equivalent chain length of 34.51 and a carbon number of 39. As predicted, the tetramethyl alkane elutes at about the same time as an internally branched dimethyl alkane with a backbone of one carbon longer (13,17-dimethyl C_{34}) (Nelson et al., 1988). The predicted α-fragment ions as indicated on the structure in Fig. 5 are all present. Neither a molecular ion nor an M-15 ion is present, but the abundance of this compound is relatively low and such ions would be of low intensity at best.

The Dufour gland chemistry of *P. cerealellae* is also dominated by hydrocarbons, but unlike the other three species, the Dufour gland of *P. cerealellae* also contains two long-chain aldehydes (Table 4, Fig. 6). In general, the hydrocarbons are the same as those found on the cuticle of this species (Howard, 2001) and consist of a series of *n*-alkanes (C_{27}–C_{31}); 3-, 4-, 5-, 7-, 8-, 9-, 11-, 12-, 13-, 14-, 15-, 16- and 17-methyl alkanes; 3, 7-, 3,9-, 3,11- and 3,13-dimethyl alkanes; 5,9-dimethyl C_{27} and 5,13-, 5,15- and 5,17-dimethyl C_{35}; 7,11-, 9,13-, 11,15-, 11,16-, 11,17-, 11,18-, 11,19-, 11,20-, 11,21-, 12,16-, 13,17-, 13,19-, 13,21-dimethyl alkanes. The monomethyl alkanes are the dominant components (ca. 57% of the total), followed by the dimethyl alkanes (ca. 32% of the total) and then by the *n*-alkanes (ca. 5% of the total). The hydrocarbons specific to the Dufour gland are the 5,9-dimethyl C_{27}, the three 5,X-dimethyl C_{35}, the 12- and 14-methyl C_{36}, the two dimethyl C_{36}, 13-methyl C_{37} and 13,17-dimethyl C_{37}. Two long-chain aldehydes are present in the Dufour gland and are unique to this species, representing ca. 4% of the total: *n*-tetracosanal (C_{23}CHO) and *n*-hexacosanal (C_{25}CHO). Fig. 7 is the EI-mass spectrum of the hexacosanal and Fig. 8 is that of the *N,N*-dimethyl hydrazone of this aldehyde. As expected, the largest high mass ion present in the spectrum of the free aldehyde is the M-18 ion fragment at m/z 362, although a very weak parent ion at m/z 380 is also present. No evidence for any methyl branches is present in this mass spectrum. The mass spectrum of the *N,N*-dimethyl hydrazone has a prominent parent ion at m/z 422, thus confirming the carbon number assignment of the free aldehyde. The base peak at m/z 86 is the diagnostic McLafferty rearrangement ion for the indicated structure (McDaniel and Howard, 1985). No aldehydes are found as cuticular components in this species (Howard, 2001).

4. Discussion

4.1. Morphology of parasitoid Dufour glands

The Dufour gland is an exocrine gland associated with the venom apparatus in all aculeate

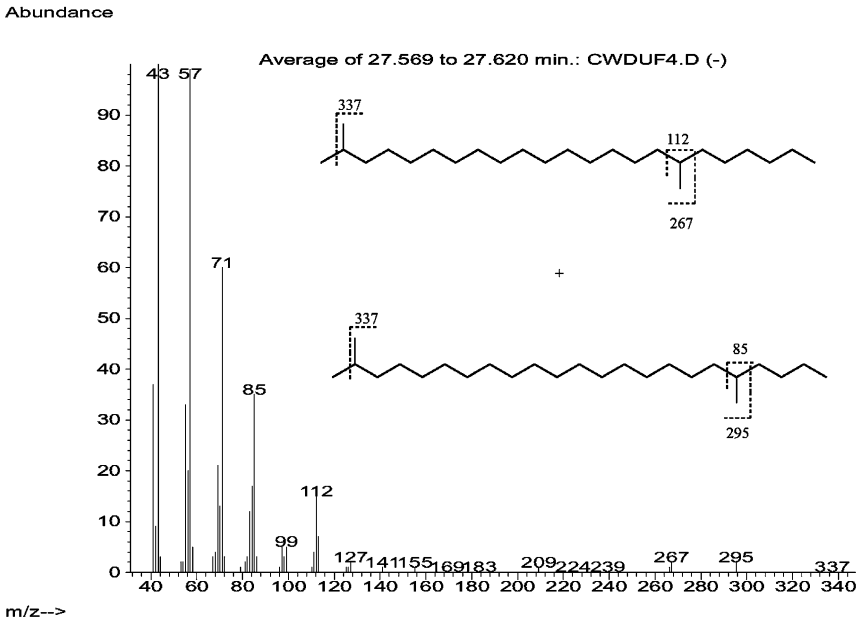

Fig. 3. EI-mass spectrum of 2,17- and 2,19-dimethyl C_{23} isolated from the Dufour gland of *C. waterstoni*.

Hymenoptera, including ants, bees, wasps and parasitoids (Robertson, 1968; Quicke, 1997; Cruz-Lopez et al., 2001). Generally, the gland is a simple ectodermally derived tube or pear-shaped sac composed of a single layer of epithelial cells, although atypically-shaped glands are found in some taxa (Billen et al., 2000). Dufour glands in the four parasitoids examined in the current study are typical in that they are simple glands, pear-shaped with a long stalk in the bethylids, and elongated, tubular to cylindrically-shaped with a short stalk in the pteromalids. Ultrastructural studies have been carried out on at least one ichneumonid, *Pimpla turionellae* (Blass and Ruthmann, 1989).

4.2. Chemistry of parasitoid Dufour glands

Unlike the aculeate Hymenoptera, where a rich literature exists on the chemistry and function of Dufour gland constituents (Ali and Morgan, 1990; Billen and Morgan, 1998), the literature on parasitoid Dufour gland chemistry is sparse, with none existing for members of either the Bethylidae or Pteromalidae. Only two species seem to have been characterized: *Venturia canescens* (Gravenhorst) (Ichneumonidae) (Mudd et al., 1982; Marris et al., 1996) and *Cardiochiles nigriceps* (Viereck) (Braconidae) (Syvertsen et al., 1995). The ichneumonid chemicals are simple mixtures of saturated and mono-unsaturated hydrocarbons: Z-8-, Z-9, and Z-10 heneicosene, heneicosane, Z-10-tricosene, tricosane, Z-10-pentacosene and pentacosane with the Z-10-tricosene being the dominant component (62% of the total mixture). The braconid Dufour gland chemistry is also composed solely of hydrocarbons, but the carbon number range is substantially greater (23 to 35) than that of the ichneumonid and consists of a homologous series of *n*-alkanes, Z-monoenes and Z,Z-alkadienes, which are also reported to occur on the cuticle of this species. The dominant components of *C. nigriceps* appear to be *n*-pentacosane, Z-13- and Z-14-nonacosene and Z,Z-7,15-nonacosadiene, since the authors state that the female Dufour gland and cuticular composition are similar. The semiochemical function of the ichneumonid Dufour gland secretions has been shown to be that of a conspecific egg marking pheromone designed to prevent superparasitism (Marris et al., 1996), whereas the semiochemical function of the braconid Dufour gland secretion has been shown to be courtship mediation (Syvertsen et al., 1995).

The Dufour gland volatile chemistry of the two bethylids and two pteromalids that we have examined are also dominated by hydrocarbons. In comparison to the chemistry of the ichneumonid and the braconid above, the bethylids and pteromalids

Table 3
Dufour gland chemistry and percentage composition in *A. calandrae*

Compound	CN[1]	ECL[2]	Diagnostic ion fragments, m/z	Mean percent (S.D.)($N=3$)
7-MeC_{29}	30	29.32	113, 337, 407	0.16 (0.12)
5-MeC_{29}	30	29.42	85, 365, 407	0.04 (0.03)
3-MeC_{29}	30	29.64	393, 407	0.02 (0.01)
Unk1	–	29.72		0.01 (0.00)
n-C_{30}	30	30.00	422	Tr
3,7-DiMeC_{29}	31	30.04	127, 337, 407, 421	0.09 (0.03)
Unk2	–	30.28		0.02 (0.01)
Unk3	–	30.87		0.29 (0.09)
11-, 13-, 15-MeC_{31}	32	31.25	169, 309; 197, 281; 225, 253; 435	0.76 (0.19)
9-MeC_{31}	32	31.30	141, 337, 435	2.96 (0.12)
7-MeC_{31}	32	31.35	113, 365, 435	3.30 (0.57)
5-MeC_{31}	32	31.40	85, 393, 435	0.64 (0.23)
13,17-DiMeC_{31}	33	31.50	197, 295, 225, 267, 449	0.43 (0.16)
9,13-DiMeC_{31}	33	31.55	141, 351, 211, 281, 449	0.20 (0.08)
7,11-DiMeC_{31}	33	31.62	113, 379, 183, 309, 449	0.35 (0.09)
3-MeC_{31}	32	31.68	421, 435	4.93 (1.76)
5,9-DiMeC_{31}	33	31.74	85, 407, 155, 337, 449	0.58 (0.03)
n-C_{32}	32	32.00	450	0.06 (0.02)
3,7-DiMeC_{31}	33	32.05	435, 127, 365, 449	3.01 (0.83)
14-, 15-MeC_{32}	33	32.23	211, 281; 225, 267; 449	0.55 (0.18)
3,7,11-, 3,9,15-TriMeC_{31}	34	32.28	449, 127, 379, 197, 309; 449, 155, 351, 253; 463	0.77 (0.19)
14,18-DiMeC_{32}	34	32.48	211, 295, 225, 281, 463	0.59 (0.14)
3-MeC_{32}	33	32.65	435, 449	0.19 (0.04)
Unk4	–	32.90		0.38 (0.16)
11-, 13-, 15-, 17-MeC_{33}	34	33.29	169, 337; 197, 309; 225, 281; 253; 463	9.26 (0.77)
7-MeC_{33}	34	33.38	113, 393, 463	6.16 (2.17)
13,17-, 15,19-DiMeC_{33}	35	33.53	197, 323, 253, 267; 477	9.20 (0.92)
7,11-DiMeC_{33}	35	33.64	113, 407, 183, 337, 477	2.37 (0.36)
3-MeC_{33}	34	33.71	449, 463	4.54 (1.63)
5,9-DiMeC_{33}	35	33.76	85, 435, 155, 365, 477	3.81 (0.71)
Unk5	–	33.85		0.54 (0.07)
C_{34}	34	34.00	478	2.34 (0.39)
3,11-DiMeC_{33}	35	34.05	463, 183, 337, 477	2.85 (0.74)
3,7-DiMeC_{33}	35	34.07	463, 127, 393, 477	0.53 (0.14)
12-, 13-, 14-, 15-MeC_{34}	35	34.23	183, 337; 197, 323; 211, 309; 225, 295; 477	1.04 (0.17)
3,7,11-TrimeC_{33}	36	34.29	477, 127, 407, 197, 337, 491	2.13 (0.28)
13,17-DiMeC_{34}	36	34.47	197, 337, 267, 491	1.24 (0.30)
3,7,11,15-TetraMeC_{33}	37	34.51	491, 127, 421, 197, 351, 267, 281, 505	3.53 (1.46)
13-, 15-, 17-MeC_{35}	36	35.25	197, 337; 225, 309; 253, 281; 491	4.71 (0.68)
13,17-DiMeC_{35}	37	35.55	197, 351, 267, 281, 505	19.05 2.34)
5,17-DiMeC_{35}	37	35.72	85, 463, 267, 281, 505	1.02 (0.29)
5,9-DiMeC_{35}	37	35.84	85, 463, 155, 393, 505	0.43 (0.09)
n-C_{36}	36	36.00	506	0.48 (0.13)
13-, 14-MeC_{36}	37	36.20	197, 351; 211, 337; 505	0.20 (0.08)
14,18-DiMeC_{36}	38	36.43	211, 351, 281, 519	1.01 (0.33)
3,7,11,15-TetraMeC_{35}	39	36.49	519, 127, 449, 197, 379, 267, 309, 533	0.57 (0.35)
13-, 15-, 17-MeC_{37}	38	37.23	197, 365; 225, 337; 253, 309; 519	0.08 (0.05)
13,17-, 15,19-DiMeC_{37}	39	37.47	197, 379, 267, 309; 225, 351, 281, 295; 533	1.67 (0.49)
Unk6	–	37.60		0.86 (0.75)
n-C_{38}	38	38.00	534	0.03 (0.04)

[1] Carbon number.
[2] Equivalent chain length.

have a considerably greater diversity of hydrocarbon classes. In particular, the ichneumonid and braconid both possessed only *n*-alkanes and mono- and dienes, whereas the two bethylids examined here contain *n*-alkanes, isomeric monoenes, 2-, 3- and 5-methyl alkanes and 5,X-dimethyl alkanes

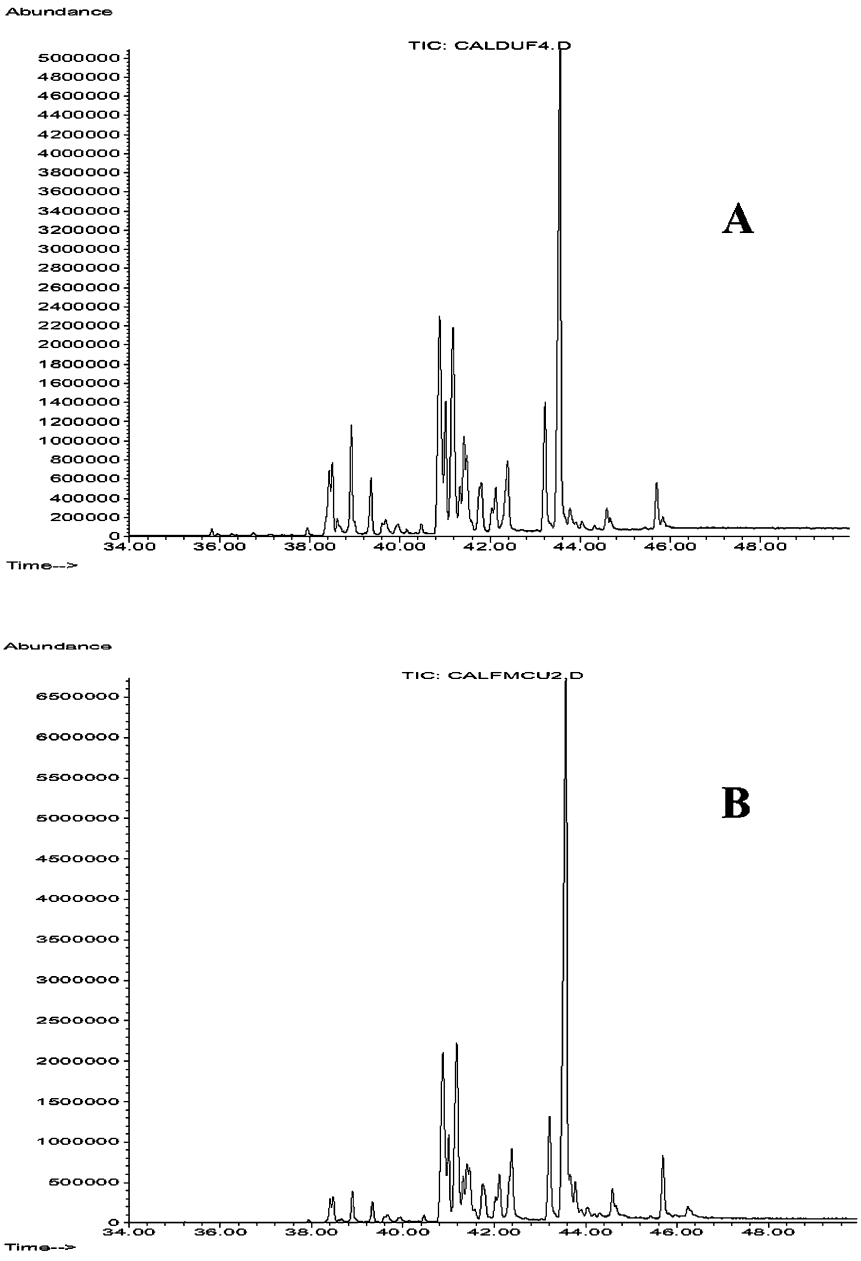

Fig. 4. Total ion trace of contents of the Dufour gland and cuticle of *A. calandrae*. (a) Dufour gland; (b) cuticle (SPME analysis).

(where X is a methyl branch located towards the interior of the alkyl chain). In addition, one of the bethylids, *C. waterstoni*, has 2,X-dimethyl alkanes, where X is 15 or 17 methylene units beyond the 2-methyl branch point. In contrast to all of the other parasitoid taxa examined, the two pteromalids examined have no unsaturated hydrocarbons. Rather, they have complex mixtures of *n*-alkanes, monomethyl alkanes with the methyl branch both close to the terminus of the carbon chain and located interiorly, a variety of classes of dimethyl alkanes, trimethyl alkanes and even tetramethyl alkanes. *P. cerealellae* has in addition, two long chain aldehydes.

To a large extent, the chemicals found in the Dufour glands of the species that we have examined are also found on their cuticle, although several specific hydrocarbons in the Dufour glands

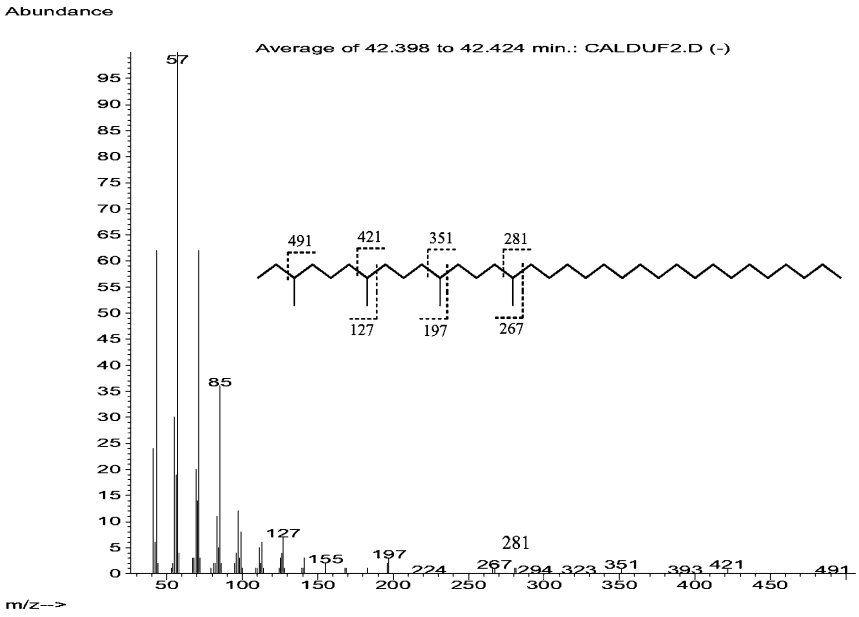

Fig. 5. EI-mass spectrum of 3,7,11,15-tetramethyl C_{33} isolated from the Dufour gland of *A. calandrae*.

of *C. waterstoni*, *A. calandrae* and *P. cerealellae* are not found as part of the cuticular complex and neither are the aldehydes from *P. cerealellae*. The relative abundance or ratios of the hydrocarbons found in both Dufour gland and cuticular extracts for any given species are similar, suggesting that they perhaps arise from a common source. A similar correspondence in Dufour gland chemistry and cuticular chemistry has also been reported for several species of bumblebees (Oldham et al., 1994), in polistine wasps (Dani et al., 1996), and in the honeybee *Apis mellifera* (Gozansky et al., 1997). The bumblebees are particularly interesting, because in several species the Dufour gland contains, besides the hydrocarbons, various oxygenated compounds, which are not found on the cuticle (Oldham et al., 1994). Ants, however, while being shown to have a large repertoire of hydrocarbons in their Dufour gland secretions (Billen and Morgan, 1998), contain mostly short- to- medium length hydrocarbons that are not cuticular components.

To date, no evidence has been obtained to suggest that the Dufour gland is the site of hydrocarbon biosynthesis. Rather, all evidence suggests that the hydrocarbons on both the cuticle and in specialized glands such as the Dufour gland arise from oenocytes, which release the hydrocarbons into the hemolymph. They are transported by lipophorin and released either across the cuticular membranes or released across the membranes of the specialized glands (Van der Horst et al., 1993; Blomquist et al., 1998; Schal et al., 1998; Jurenka and Subchev, 2000). Gozansky et al. (1997) directly showed that in vitro incubation of the Dufour gland of the honeybee with 1-C^{14}-acetate did not result in biosynthesis of hydrocarbons in this gland, whereas injection of the labelled acetate into the whole organism and then isolating Dufour gland hydrocarbons did result in uptake of the label. These studies, however, still beg the question of where the hydrocarbons found in the Dufour gland of *C. waterstoni*, *A. calandrae* and *P. cerealellae*, but not found on the cuticle of these species, originate. If they are produced by oenocytes, then some mechanism must exclude them from transport to the cuticle, while allowing selective uptake by the Dufour gland cell membranes (Schal et al., 1998). Further, studies will be required to solve this dilemma.

The semiochemical function, if any, of the constituents of the Dufour glands of *C. tarsalis*, *C. waterstoni*, *A. calandrae* and *P. cerealellae* also remain to be discovered. It seems a fair assumption that given the biochemical investment that the females have put into the production of the relatively substantial quantities of these chemicals that they must serve some useful function in the eco-

Table 4
Dufour gland chemistry and percentage composition in *P. cerealellae*

Compound	CN[1]	ECL[2]	Mean% (S.D.)($N=3$)
n-tetracosanal ($C_{23}CHO$)[3]	24	–	1.78 (0.57)
n-C_{27}	27	27.00	0.35 (0.07)
9-MeC_{27}	28	27.32	0.20 (0.00)
7-MeC_{27}	28	27.40	0.05 (0.02)
5-MeC_{27}	28	27.46	0.16 (0.01)
3-MeC_{27}	28	27.69	0.61 (0.12)
5,9-$DiMeC_{27}$[4]	29	–	0.12 (0.01)
n-C_{28}	28	28.00	0.11 (0.03)
3,7-$DiMeC_{27}$	29	28.01	0.15 (0.01)
n-hexacosanal ($C_{25}CHO$)[5]	26	–	2.29 (0.29)
12-MeC_{28}	29	28.30	0.08 (0.02)
5-MeC_{28}	29	28.47	0.06 (0.01)
3-MeC_{28}	29	28.70	0.40 (0.04)
n-C_{29}	29	29.00	2.65 (0.62)
9-, 11-, 13-MeC_{29}	30	29.30	3.70 (0.27)
7-MeC_{29}	30	29.40	0.46 (0.19)
5-MeC_{29}	30	29.46	0.20 (0.04)
9,13-, 7,11-$DiMeC_{29}$	31	29.61	1.38 (0.31)
3-MeC_{29}	30	29.73	14.09 (2.75)
n-C_{30}	30	30.00	0.28 (0.02)
3,7-$DiMeC_{29}$	31	30.01	2.42 (0.24)
11-, 12-, 13-, 14-, 15-MeC_{30}	31	30.30	0.66 (0.07)
8-MeC_{30}	31	30.33	0.36 (0.06)
4-MeC_{30}	31	30.61	0.94 (0.12)
3-MeC_{30}	31	30.70	0.83 (0.10)
n-C_{31}	31	31.00	1.75 (0.19)
11-, 13-MeC_{31}	32	31.29	6.94 (0.29)
11,15-, 11,17-, 11,19-$DiMeC_{31}$	33	31.61	2.59 (0.21)
9,13-, 9,15-$DiMeC_{31}$	33	31.70	2.34 (0.35)
3-MeC_{31}	32	31.74	8.72 (1.54)
Unk1	–	–	0.29 (0.08)
3,7-, 3,9-$DiMeC_{31}$	33	32.05	2.12 (0.29)
11-, 12-, 14-, 16-MeC_{32}	33	32.29	1.27 (0.25)
8-MeC_{32}	33	32.33	0.90 (0.21)
11,16-, 11,18-, 11,20-$DiMeC_{32}$	34	32.61	1.93 (0.29)
Unk2			0.21 (0.05)
11-, 13-, 15-, 17-MeC_{33}	34	33.30	9.60 (0.69)
11,17-, 11,19-, 11,21-$DiMeC_{33}$	35	33.61	11.51 (1.24)
3-MeC_{33}	34	33.74	1.94 (0.20)
Unk3	–	–	0.45 (0.19)
3,7-, 3,9-, 3,11-, 3,13-$DiMeC_{33}$	35	34.05	0.82 (0.15)
12-, 14-MeC_{34}	35	34.30	0.92 (0.20)
8-MeC_{34}	35	34.33	0.51 (0.13)
12,16-, 13,17-, 14,18-$DiMeC_{34}$	36	34.60	0.97 (0.24)
11-, 13-MeC_{35}	36	35.30	2.69 (0.08)
13,17-, 13,19-, 13,21-$DiMeC_{35}$	37	35.61	4.95 (0.71)
5,13-, 5,15-, 5,17-$DiMeC_{35}$[6]	37	35.68	0.44 (0.20)
Unk4	–	–	0.14 (0.08)
Unk5	–	–	0.24 (0.09)
12-,14-MeC_{36}[7]	37	36.30	0.09 (0.02)
12,16-, 13,17-$DiMeC_{36}$[8]	38	36.60	0.21 (0.06)
13-MeC_{37}[9]	38	37.30	0.15 (0.04)
13,17-$DiMeC_{37}$[10]	39	37.61	0.96 (0.20)

[1] Carbon number.
[2] Equivalent chain length.
[3] Diagnostic EI-MS ions: m/z 334 (M-18), 352 (M^+); m/z 86, 394 (M^+) dimethyl hydrazone.
[4] Diagnostic EI-MS ions: m/z 85, 351, 155, 281.
[5] Diagnostic EI-MS ions: m/z 362 (M-18), 380 (M^+); m/z 86, 422 (M^+) dimethyl hydrazone.
[6] Diagnostic EI-MS ions: m/z 85, 477, 211, 337;85, 477, 281, 295;85, 477, 225, 323.
[7] Diagnostic EI-MS ions: m/z 183, 365; 211, 337.
[8] Diagnostic EI-MS ions: m/z 183, 379, 253, 309; 197, 365, 267, 295.
[9] Diagnostic EI-MS ions: m/z 197, 365.
[10] Diagnostic EI-MS ions: m/z 197, 379, 267, 309.

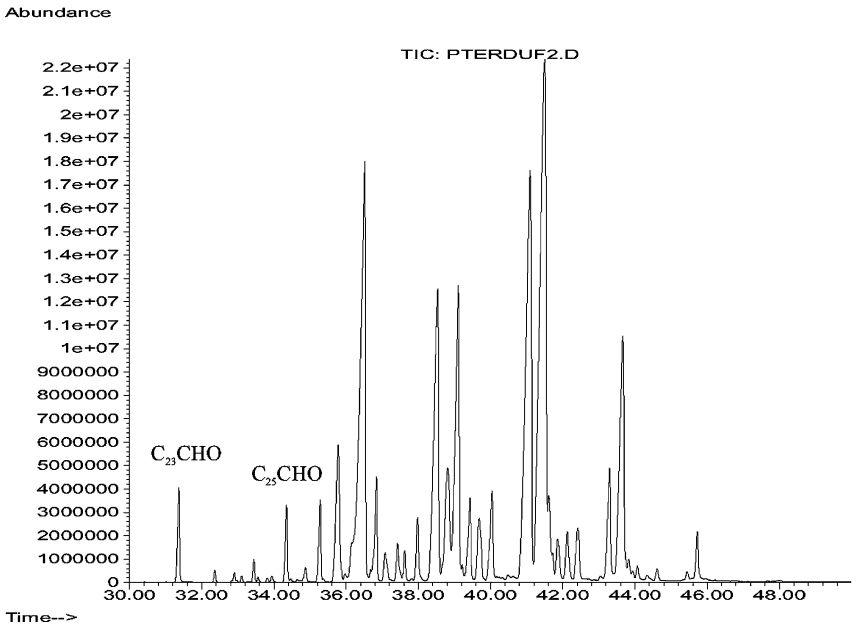

Fig. 6. Total ion trace of contents of the Dufour gland of *P. cerealellae*.

logical relationships of the species. The non-cuticular components that we detected in the Dufour glands of the bethylids and pteromalids, particularly the long-chain aldehydes in *P. cerealellae*, are certainly prime candidates for a semiochemical function in these parasitoids. As noted earlier, the Dufour gland components of the parasitoid *C. nigriceps* were postulated to serve as sex pheromones (Syvertsen et al., 1995), whereas those of *V. canescens* were suggested to serve a role in

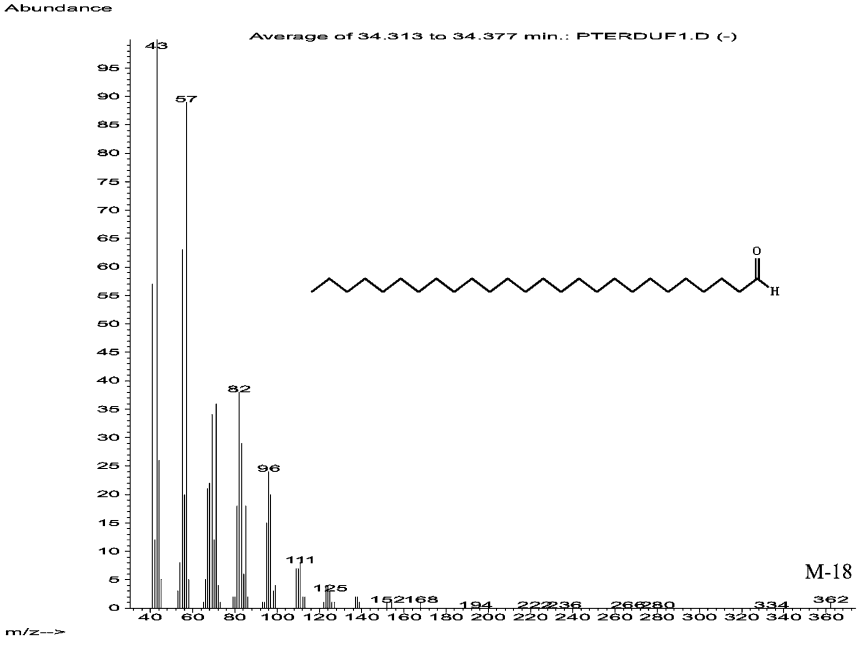

Fig. 7. EI-mass spectrum of *n*-hexacosanal isolated from the Dufour gland of *P. cerealellae*.

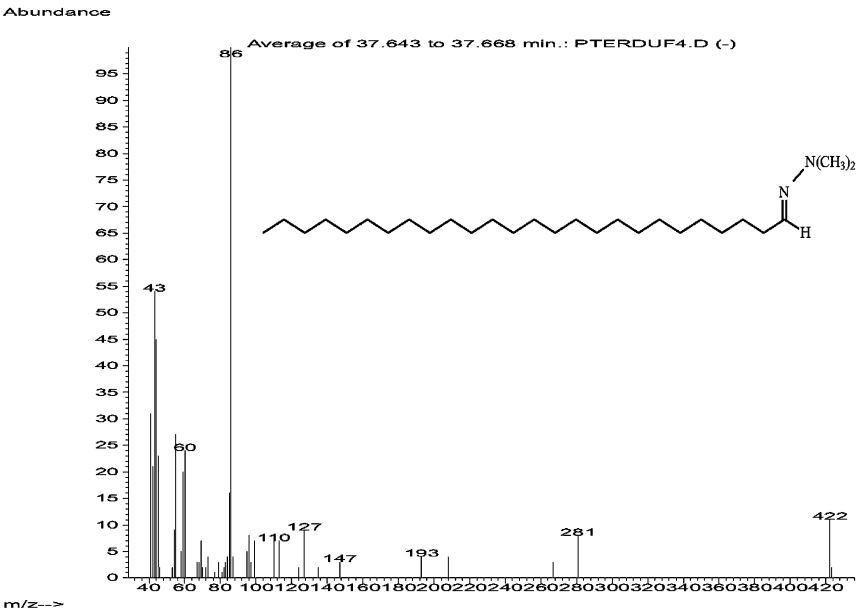

Fig. 8. EI-mass spectrum of the *N,N*-dimethyl hydrazone of *n*-hexacosanal isolated from the Dufour gland of *P. cerealellae*.

preventing superparasitism by closely related females (Marris et al., 1996). Both of these species are endoparasitoids, whereas all the species we examined are ectoparasitoids. Bender (1943) postulated that the function of the Dufour gland secretion in *Habrobracon juglandis* was to serve as a lubricant in aiding the passage of the egg along the ovipositor. Robertson (1968) and Copland and King (1971) have also postulated a similar possible function, although the postulate has never been experimentally confirmed. Hoffmeister (2000) postulated that the pteromalid parasitoid *Halticoptera rosea* applied a marking pheromone, derived either from the wasp's Dufour gland or poison gland to the surface of rose hips in which the fly host, *Rhagoletis basiola*, had deposited its eggs in the fruit pulp. This marking pheromone was shown to deter further oviposition by conspecifics. Considerable evidence now exists that cuticular hydrocarbons function in some cases as species and gender recognition cues (Howard, 1993) and it is possible that the Dufour gland hydrocarbons may be serving a similar role. If parasitoids use even a fraction of the semiochemical roles postulated for the aculeate Hymenopteran Dufour gland components (Ali and Morgan, 1990; Billen and Morgan, 1998) then numerous other roles than the ones identified to date are likely to be found for these secretions.

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